Immunohistochemical Localization of μ-Opioid Receptors in Human Dental Pulp

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Abstract

Studies in both animal and clinical models suggest that opioids exert their analgesic effects not only through activation of receptors in the CNS, but also through interaction with peripheral opioid receptors. The present study evaluated the presence and distribution of μ-opioid receptors in human dental pulp. Human third molars indicated for extraction were removed, fixed in 4% paraformaldehyde and 0.2% picric acid, and decalcified in 10% EDTA and 7.5% PVP. The teeth were cut using a cryostat and the avidin-biotin peroxidase immunohistochemistry technique used. Immunostaining for μ-opioid receptors was detected along the nerve bundle of the radicular as well as coronal dental pulp. Positive immunostaining was also observed in the individual nerve fibers in the coronal region. This demonstration of opiate receptors on pulpal nerve fibers suggests a peripheral site in the dental pulp where endogenous or exogenous opioids can interact with μ-opioid receptors.

Introduction

Exogenous opioids derived from plant alkaloids such as morphine and its derivatives are used to control different types of pain in human, presumably by direct action of opioids at specific receptors within the central nervous system (1). Based on pharmacological studies, at least three classes of receptors have been defined termed μ, δ, and κ (2). However, morphine and most clinical opioid analgesics seems to act through μ-opioid receptors (3).

A historical report by Wood (4) suggested that morphine induces an analgesic effect when applied to a painful area in the periphery. Recently, it has been demonstrated that both endogenous (5), and exogenous (6,7) opioid agonists produce peripheral antinociception effects by interaction with peripheral opioid receptors in rat tissue. The three types of opioid receptors (μ, δ and κ) have been shown to be present in the peripheral sensory nerves and can modulate both afferent and efferent neuronal functions (8,9). In addition, the gene expressing μ-opioid receptor has been detected in peripheral rat tissues in the small and large intestine, liver, adrenal, kidney, lung, spleen, testis, ovary, and uterus (10).

Consistent with the identification of opioid receptors in the peripheral nervous system are clinical trials demonstrating analgesic

effects of locally applied opioids. Analgesia is induced following peripheral administration of low dose morphine by periodontal ligament injection to chronically inflamed teeth (11). Furthermore, consistent results have come from studies of the intra-articular application of small, systemically inactive doses of morphine during knee surgery (12), and a study which demonstrated that 1 mg of morphine added to local anesthetic results in postoperative analgesia at 8-12 hours following oral surgery (13).

Demonstration of opiate analgesia when administrated peripherally to inflamed teeth, the presence of opiate receptors in other tissues sensitive to peripheral effects of opiates and the known action of Morphine and most clinical opioid analgesics through μ-opioid receptors, prompted us to examine human dental pulp for the presence of μ-opioid receptors.

Here we present results from immunohistochemical study for the presence of μ-opioid receptor along the nerve bundle and in individual nerve fibers of the human dental pulp. These results provide the basis for further studies to explore a functional relationship between these receptors and peripheral opiate analgesia in the trigeminal system.

Methods

Fully erupted caries-free human third molars (total number: 10) were extracted under local anesthesia from different subjects who granted informed consent for the study which was approved by the NIDCR Institutional Review Board. Immediately after tooth extraction, the pulp chamber was exposed by perforating the tooth at the coronal region using a round surgical bur. Pulp fixation was performed immediately by immersion of the tooth in 4% paraformaldehyde and 0.2% picric acid, followed by decalcification in 10% ethylenediaminetetraacetic acid (EDTA) prepared in PBS (pH 7.3) and 7.5% polyvinylpyrrolidone (PVP) at 4°C. The teeth were cut into 20-μm thick longitudinal sections using a cryostat (Leitz, 1720 Digital), and then processed for immunohistochemistry using the classic avidin-biotin peroxidase technique.

Briefly, endogenous peroxidase activity was eliminated by incubating the sections for 30 minutes in 0.3% H_2O_2 in methanol at room temperature, followed by washing in phosphate buffered saline (PBS) at pH 7.3 for 30 minutes at room temperature. In order to block the non-specific staining, sections were incubated for 20 minutes with normal goat serum (Vector Laboratories, California, USA). Excess serum was blotted from sections before incubation at 4°C for overnight with the primary

antibody against μ-opioid receptor (dilution 1:900) which was developed in rabbit using MOR peptide (Incstar Corporation, Minnesota USA). For control sections, the primary antibody was replaced by either PBS, normal rabbit serum, or with μ-opioid receptor (MOR) antibody which has previously pre-incubated with MOR peptide (Incstar Corporation, Minnesota USA).

Sections were then washed for 30 minutes in PBS before and after incubation with diluted biotinylated secondary antibody solution (Vector Laboratories, California, USA), and Vectastin ABC reagent for 30 minutes each, respectively (Vector Laboratories, California, USA).

Finally, the sections were incubated with peroxidase substrate solution (Vector Laboratories, California, USA) until desired stain intensity was developed. Sections were then rinsed in tap water for 5 min. and then mounted without counterstaining. Another two groups of identically prepared sections were stained with either silver staining (Nauta Gygax method) to detect the nerve fibers or with hematoxylin. The sections were then examined by light microscopy (Nikon-Mocrophot-FX) and photographed with Kodak (Elite Chrome, ASA 200) slide film.

Results

Immunostaining for μ-opioid receptors was detected in both the coronal and radicular region of human tooth pulp. Immunostaining for μ-opioid receptors was detected along the individual nerve fibers located in the pulp chamber (Fig. 1 A, B). Positive immunostaining was also observed in the nerve bundle of the radicular (Fig. 1 E) as well as coronal dental pulp.

Control experiments were performed using adjacent sections substituting the primary antibody with either PBS, normal rabbit serum, or with μ-opioid receptor antibody previously incubated with MOR peptide (the antigen used to produce the μ-opioid receptor antibody in the rabbit). These control sections showed no detectable immunoreaction along the individual nerve fibers or nerve bundles found in the coronal or radicular region of the dental pulp (Fig. 1 C, D).

Discussion

The present study demonstrates the presence of μ-opioid receptors on the nerve fibers of the human dental pulp. This finding is consistent with previous studies that reported the presence of opioid binding sites and gene expression of μ-opioid receptor in the peripheral nervous system (10, 14).

Both animal studies and clinical trials (5-7) indicate that peripheral anti-nociceptive effects of opioid agonists are induced by interaction with peripheral opioid receptors. In the light of these studies together with our recent and present reports which demonstrated an analgesic effect of morphine when injected in the periodontal ligament of chronically inflamed teeth and the presence of μ-opioid receptor in the human dental pulp, respectively, we suggest that peripheral administration of an opioid agonist in the dental pulp might induce an anti-nociceptive effects mediated by peripheral opiate receptors.

It has been shown that inflammation upregulates peripheral opioid receptors (15,16). There is also evidence that opioid receptors on sensory nerves are inactive or inaccessible due to a perineurial barrier under normal non-inflamed conditions (17-19). Antonijenovic et al., (8) suggest that earlier stages of inflammation disrupt the perineural barrier and

facilitates access for agonists to their corresponding receptors, while in later stages of the inflammatory reaction, an increase in synthesis and peripheral axonal transport of opioid receptors occurs. This notion is consistent with the observation that peripheral opioid analgesia can be demonstrated in chronically inflamed human tissues (11). In the light of these results we hypothesize that exogenous opioids might not be accessible to opioid receptors in the dental pulp until the presence of inflammation.

It should be noted however that the analgesic effects of opioids outside the CNS offers an interesting clinical implication, since the undesirable side effects such as respiratory depression, nausea, sedation or dependence liability can be devoided, for review see Herz, 1996 (19).

The present study did not examine the type of nerve fibers associated with μ-opioid receptors in the dental pulp. However, it has been reported that small, unmyelinated, slow conducting nociceptors of the rat trigeminal ganglia are the most affected when μ-opioid receptors are activated (20), and opioid binding sites were mainly associated with small diameter nerves of the human synovia (14). In light of these findings, we hypothesized that distribution of μ-opioid receptors in the human dental pulp might be mainly associated with small diameter C-fibers rather than

larger diameter A-fibers. However, further studies are needed to clarify this possibility.

In summary, here we report the presence of μ-opioid receptors in the human dental pulp. This observation suggests a possible functional relationship between opiate receptors in dental tissues and peripheral opiate analgesia.

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Figure 1. Photomicrographs showing immunohistochemical localization of μ-opioid receptors in the human tooth pulp. A: The coronal region of the tooth pulp showing a positive immunostaining along the individual nerve fibers. B: High magnification of a nerve fiber located in the coronal region of the tooth pulp showing a positive immunostaining with μ-opioid receptor antibody. C: control section with negative immunostaining. In this section the primary antibody was substituted by μ-opioid receptor antibody previously incubated with MOR peptide. D: High magnification of a control section (C). E: Positive immunostaining with μ-opioid antibody is shown along a nerve bundle in the radicular region of a tooth pulp. Original magnifications (bars: A= 20 μm, B=10 μm, C= 50 μm, D= 10 μm, E= 50 μm).